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10/522,405	09/30/2005	Andrea Cossarizza	COSSARIZZA-1	5546

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EXAMINER	
STAPLES, MARK	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/522,405	COSSARIZZA, ANDREA	
	Examiner	Art Unit	
	Mark Staples	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 May 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 05/30/2008 has been entered.

2. Applicant's amendment of claims 1 and 24 in the paper filed on 11/02/2007 is acknowledged.

Claims 1-24 are pending and at issue.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Objections and Rejections that are Withdrawn

3. The objection to the specification is withdrawn in light of Applicant's amendment of capitalize trademarks.

Claim Rejections Withdrawn - 35 USC § 112 Second Paragraph

4. The rejection of claims 1, 3-5, 8-9, and 11-16 under 35 U.S.C. 112, second paragraph for indefiniteness for omitting an essential step is withdrawn. Applicant's amendments to claim 1 have overcome this rejection by reciting “relative copy number (CN)” in the preamble and obtaining "the relative CN" in step (3).

5. The rejection of claims 7-9 under 35 U.S.C. 112, second paragraph for indefiniteness for not redefining a common term to its contrary meaning the claim is withdrawn. Applicant's amendments to claim 1 have overcome this rejection by reciting the essential relationships of the NucSeq’s to each other.

6. The rejection of claims 17-23 under 35 U.S.C. 112, second paragraph, as being incomplete for omitting an essential step is withdrawn. Applicant's amendments to claim 1 have overcome this rejection by reciting “relative copy number (CN)” in the preamble and obtaining "the relative CN" in step (3).

7. The rejection of claim 24 under 35 U.S.C. 112, second paragraph, as being incomplete for omitting an essential step is withdrawn. Applicant's amendments to claim 24 have overcome this rejection by reciting “relative copy number (CN)” in the preamble.

8. The rejection of claims 17-23 and 24 under 35 U.S.C. 112, second paragraph, as being indefinite for not redefining a common term to its contrary meaning in the claim is withdrawn. Applicant's amendments to claim 1 and 24 have overcome this rejection by reciting the essential relationships of the NucSeq’s to each other.

9. The rejection of claims 1-24 under 35 U.S.C. 112, second paragraph, as being indefinite for lack of antecedent basis for monitored by fluorescence is withdrawn.

Applicant has amended claims 1 and 2 to provide antecedent basis fro fluorescence. However, a new rejection under 35 U.S.C. 112, second paragraph necessitated by amendment is given in regards to the "monitoring of fluorescence", please see below.

10. The rejection of claim 2 under 35 U.S.C. 112, second paragraph, as being indefinite for lack of antecedent basis for “absolute CN” is withdrawn. Applicant is correct that the claim had been amended to provide proper antecedent basis.

11. The rejection of claims 1, 3-5, 8, 11, 12, and 17-23 under 35 U.S.C. 112, second paragraph, is withdrawn as being incomplete for omitting essential structural cooperative relationships of elements of how the CN of NucSeql and the CN of NucSeqll each relate to the relative CN as given in the formula in step 1(3)of claim 1. Applicant argument is persuasive.

Rejections that are Maintained

Art Cited by Applicant Not Provided

12. Examiner is not in possession of any new references in the responses of Applicant filed on 11/02/2007 and 05/30/2008. Nor is there a new IDS filed. Therefore the references which Applicant cites within the responses for those filing dates have not been considered.

Claim Rejections Maintained - 35 USC § 112 First Paragraph

13. The rejection of claims 1-24 under 35 U.S.C. 112, first paragraph is maintained, because the specification, while being enabling for detection using probes with both quenchers and fluorophore, does not reasonably provide enablement for detection by a fluorophore alone by any measure of concentration. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The nature of the invention and breadth of claims

Claims 1-24 broadly recite any fluorescent method of monitoring without little limitation on what is fluorescing (probes are labeled) and any expression of concentration including weight per volume concnetration. However, the art teaches the use of molar concentration to determine copy numbers and relative copy numbers as taught by Ginzinger et al. (2000), Ginzinger et al. (2002), Zhang et al. (1997), and Zhang et al. (1997). Furthermore, in each of these teachings it is clear what the fluorescent moiety is and what its detection relates to and how the assay format leads to a fluorescent measurement that is related to copy number. These references also go into detail on the amplification technique used and how those techniques are suited to determining copy number.

Applicant's response does not address the specific fluorescence detection techinques of the cited prior art. The prior art teaches by example that only certain fluorescent techniques can be used in single tube assays to monitor amplification. Ginzinger et al. (2000) teach: "The amount of FAM fluorescence in each reaction liberated by the exonuclease degradation of the TaqMan probe during PCR amplification . . , (see 1st full paragraph on p. 5405). It is further noted that an exonuclease is not required for amplification and an exonuclease is not recited in the

instant claims. Therefore the instant claims are not enabled for the technique of Ginzinger et al. (2000). Ginzinger et al. (2002) also teach the exonuclease technique and a variety of other techniques all of which require not only the fluorescent label but another specific and essential component, such as a quencher, and where the additional component is not necessary for amplification (se 3rd full paragraph on p. 504). It is further noted that another essential component for detection of fluorescence, other than the fluorescent label, is not recited in the instant claims. Therefore the instant claims are not enabled for the techniques of Ginzinger et al. (2002). Zhang et al. teach use of EtBr to detect the DNA after an essential separation step on agarose gel, that is the detection is not done in the single container of claimed invention. Therefore the instant claims are not enabled for the techniques of Zhang et al. (1997).

Applicant has not provided any evidence or prior art teaching or disclosure in the specification that concentration of an oligonucleotide can be determined with just one fluorescent label in an amplification performed in a single container.

Applicant further argues that a relative copy number can be determined from the relative concentrations regardless of whether those concentrations contain information about the number of copies or not. The claimed invention is at least not enabled for using concentration of weight per volume as follows. For ease of discussion, the example used are where Conc-I_{SCI} and Conc-II_{SCII} are expressed per the same unit volume and thus the only thing varying between Conc-I_{SCI} and Conc-II_{SCII} is the weight of SCI and SCII. In this example discussion “ng” will be used for the weight. In this example the molecular weight of the oligonucleotide of the SCI is not equal to the molecular weight of the oligonucleotide corresponding to SCII (which is a broad and reasonable interpretation encompassed by the claimed invention). Thus when one has

X ng per unit volume of the SCI which is Conc-I_{SCI} and Y ng per unit volume of the SCII which is Conc-II_{SCII}, the ratio of these is:

(Conc-I_{SCI} / Conc-II_{SCII}) = (X ng per unit volume)/(Y ng per unit volume) = X/Y ,

which is the formula of instant claim 1 and 24 and which results in a weight ratio X/Y as the unit volume cancels.

Thus one knows the relative weight of X to Y and that is all. Without knowing the relationship of the weight of X to its copy number and the weight of Y to its copy number, or some other copy number relationship to the weight(s), one cannot determine the relative copy number by the formula given in instant claims 1 and 24. Multiplying the ratio by the copy number of Y simply results in the copy number of Y times the weight ratio, and does not yield the copy number of X.

It is further noted that while it is recited that NucSeqI hybridizes to the complement of NucSeqI' and vice versa and in the same manner for NucSEqII and NucSeqII', this does not establish a one-to-one relationship or any other factor of relationship of the copy number of NucSeqI to NucSeqI' and the copy number of NucSeqII to NucSeqII'. That is, these recitations allow for regions of undefined length of NucSeqI and NucSeqII' to hybridize to each other, and independently for portions of undefined length of NucSeqII and NucSeqII' to hybridize to each other. (For example, NucSeqI could hybridize twice to two separate regions of NucSeqI' having the same or partially the same nucleic acid sequence, NucSeqII could hybridize three times to three separate regions of NucSeqII' having the same or partially the same nucleic acid sequence.) Thus without a one-to-one or other known factor of relationship of hybridization and even further without the knowledge of how many labels via probes, if any labels or probes, attach per each molecule of NuqSeqI, NucSeqI', NuSeqII, and NucSeqII' and that the number of labels may vary, if present, even between NucSeqI

and NucSeqI' and may vary, if present, between NucSeqII and NucSeqII; one also does not know the relationship of the copy numbers of these sequences to each other via the claimed complementary regions. It is noted that the language "hybridize to the complement" is broadly and reasonably interpreted to allow hybridization to any regions of or up to and including the full complement.

Applicant has not provided any evidence or prior art teaching or disclosure in the specification that a relative copy number can be determined from weight per volume concentrations alone, that is without any knowledge of the relationship of the weight of a oligonucleotide to its corresponding number of copies.

That concentration of weight per volume alone is not sufficient to determine copy number without some relationship of that concentration to copy number is evidenced by Gibson et al. (1996) who teach:

> "As a general rule, the target and control should use the same primers, contain similar guanine + cytosine (G + C) content, and be of equal or similar length. Once a control has been designed, it is important to validate the internal control. Validation requires demonstration that the competitive control amplify with equal efficiency and achieve plateau simultaneously with the target (Raeymaekers 1995)" (see 1st column of text on p. 995).

Once such conditions are met, Gibson et al. further teach:

> "Although absolute quantitation requires the accurate determination of internal control concentration, relative quantitation can be established easily with a validated internal control" (see bottom of 1st column on p. 995).

It is also noted that Gibson et al. teach use of a quencher and fluorophore and further teach that the probes and probe fluorophores for the target and control are different to permit separate detection of each (see 1st full paragraph of 2nd column on p. 999 and see Table 1).

Thus the prior art teaches that there must be a structural relationship of the control to the unknown target oligonucleotides in order to determine relative copy number. The broadly claimed invention does not provide such structural relationship or adequate relationship of the controls to the target oligonucleotides.

Working Examples

In response to applicant's argument that the working example in the specification shows certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., a quencher and a fluorophore) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Thus the sole example disclosed in the specification is not recited in the claims. Applicant has no working example in the specification that demonstrates that Applicant was in possession of the broadly claimed invention.

Guidance in the Specification.

Applicant argues that the claimed invention, determining the concentration of an oligonucleotide with just one fluorescent label in an amplification performed in a single container, was well known in the art. Applicant however does not provide any citation of this and does not point to support in the specification of this.

Furthermore, the claimed invention does not recite a separation step which is an essential step in the method of Zhang et al. (1997) as already noted above.

The unpredictability of the art and the state of the prior art

1. Reliability

Examiner does not disagree with the points that Applicant's makes under this subsection. The overriding point is that the claimed invention is not limited to the points that Applicants makes but is to a much more broadly claimed invention of determining copy number using concentration of an oligonucleotide which can be expressed in weight per volume and where the concentration is quantitated with just one fluorescent label in an amplification performed in a single container.

Thus the difficulties in reliability of the methods as taught in the art only make the broadly claimed method more unreliable, if in fact the broadly claimed invention is possible.

2. Many Biological Samples Contain Inhibitors.

Examiner agrees with this point and recognizes the quotation by Bustin which is given.

3. Normilization

The normalization of an amplification reaction in regards to concentrations of amplified products (and without regard to copy number) was well known. But the broadly claimed method does not provide the necessary essential steps and/or essential elements to achieve normalization of copy number. It is unclear, as noted above, as to how the broadly claimed method determines copy number. No claim recitation is given as to how the known copy number of the internal standard relates to the determine concentration of an unknown copy number of an unknown

oligonucleotide, especially in consideration of the permutations possible in the probe hybridization, the permutations possible in the number of labels per probe, the permutations possible in the number and specificity of probes, and conducting the amplification reaction in a single container as already discussed. As already noted and confirmed by Applicant in response, such amplification is not a trivial matter and the cited art goes into specific and great detail so that the art-cited amplifications can be performed and reproduced.

Quantity of Experimentation

Examiner disagrees with Applicant's conclusion that a skilled artisan would know how to carry out the amplification reaction as broadly claimed to determine relative copy number. Art recognized parameters that need to be specified are already given in the previous Office Action.

It is noted that the features upon which applicant relies (i.e., competitive and non-competitive formats) to enable the claims are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Guidance in the Specification.

Applicant is correct that the prior statement of Examiner needs clarification. Examiner amends his prior statement as follows. The specification provides no evidence that monitoring fluorescence of any single component would be feasible to determine relative copy number (the amendment is underlined). As stated in the beginning of this section, the issue is one of scope of enablement. If properly claimed,

the invention may be enabled for detection using probes with both components of a quencher and a fluorophore. Depending on how this is claimed, the lack of enablement for determining copy number may or may not be overcome as well.

Level of Skill in the Art

To use the broadly claimed invention, the level of skill in the art is deemed to be high. Applicant does not present argument against this.

Conclusion

The conclusion of the prior Office action is maintained. The broadly claimed methods are not enabled.

New Rejections Necessitated by Amendment

Claim Rejections - 35 USC § 112

14. Claims 1-24 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

15. Claims 1-24 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential structural cooperative relationships of elements, such omission amounting to a gap between the necessary structural connections. See MPEP § 2172.01. The omitted structural cooperative relationships are: the relationship of the fluorescently labeled probes to any or all of the sequences NucSeqI, NucSeqII, NucSeqI', and/or NucSeqII' as recited in claims 1 and 24. Dependent claims 2-23 are thus also indefinite.

16. Claims 1-24 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: the step(s) of amplification "monitored by flourescence" which allow "determining from the results of amplification of step (2) the concentration of NucSeqI and NucSeqII" as recited in claims 1 and 24 and how such amplification "monitored by amplification" relates to the standard curve of SC_1 and SC_2.

Prior Art

17. No prior art was found which teaches or fairly suggests a nucleic acid amplification technique that uses two nucleic acid sequences on a single vector as

controls to determine the relative copy number ratio of two corresponding nucleic acid sequences. The closest prior art found was Ginzinger et al. (2002), Zhang et al. (1997), and Gibson et al. (1996) each of whom teach use of known nucleic acid sequences to determine relative copy numbers of unknown nucleic acid sequences. However, none of Ginzinger et al. (2002), Zhang et al. (1997), or Gibson et al. (1996) teach or fairly suggest a control or standard which has two nucleic acid sequences on a single vector.

Conclusion

18. Claims 1-24 are rejected.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark Staples whose telephone number is (571) 272-9053. The examiner can normally be reached on Monday through Thursday, 9:00 a.m. to 6:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Mark Staples
/M. S./
Examiner, Art Unit 1637
July 29, 2008

/Kenneth R Horlick/

Primary Examiner, Art Unit 1637